



Supplementary Figure S4. Correlation between axon quantification methods. Optic nerves were obtained and processed from 6 male Wistar rats with laser-induced ocular hypertension as described in the methods section. Retinal ganglion cell axons were quantified using three methods: 1) the quantitative optic nerve damage grading method used in this study, 2) quantification of axons in 100% of the optic nerve cross sectional area (the gold standard) and 3) quantification of axons in a random sample of ten 630x fields comprising approximately 20% of the optic nerve. There was a very strong, positive correlation between retinal ganglion cell axon loss measurements obtained with our method of quantitative nerve damage grading compared to full axon counts of the entire optic nerve cross section (slope 0.94 ± 0.09 , Y-intercept 1.94 ± 4.76 , $R^2 = 0.96$, Panel A). There was also a positive (though weaker) correlation between the sampling method when compared to full axon counts (slope 0.91 ± 0.30 , Y-intercept 9.20 ± 15.11 , $R^2 = 0.69$, Panel B). Graphs show a best-fit linear regression depicted as a solid line with 95% confidence intervals for the fit depicted as dotted lines.